

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



PATENT ABSTRACTS OF JAPAN

(11) Publication number: **06082423 A**

(43) Date of publication of application: 22 . 03 . 94

(51) Int. Cl

G01N 27/447(21) Application number: **04257370**(71) Applicant: **SHIMADZU CORP**

(22) Date of filing: 31 . 08 . 92

(72) Inventor: **ARAI AKIHIRO**

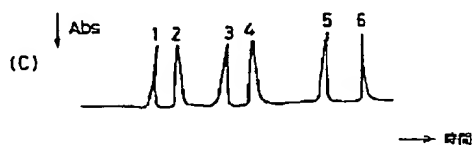
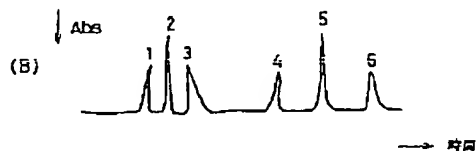
(54) **CAPILLARY ELECTROPHORESIS ANALYTICAL
METHOD BY INDIRECT ABSORBANCE
DETECTING METHOD**

COPYRIGHT: (C)1994,JPO&Japio

(57) Abstract:

PURPOSE: To improve separation between peaks of sample ionic species and to improve detection sensitivity.

CONSTITUTION: In a chart (B), 2 components of UV absorber ions are used as background electrolytes, and one of the ion is close to a component ion of a peak 2 while the other ion is close to a component ion of a peak 5. The peak 2 and the peak 5 are symmetrical, and a peak 1 and a peak 4 have slight fronting while a peak 3 and a peak 6 have slight tailing. in a chart, (C), 3 components of UV absorber ions are used as background electrolytes, and each effective mobility lies between sample components of a peak 1 and a peak 2, between sample components of a peak 3 and a peak 4, and between sample components of a peak 5 and a peak 6. The peaks 1-6 have approximately symmetric forms respectively, and sharp peaks over the wide range of mobility can be obtained.



NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

CLAIMS

Claim(s)]

Claim 1] The background electrolyte which has absorption in carrier electrolyte liquid at detection wavelength is included using capillary-electrophoresis equipment equipped with the ultraviolet detector. In the capillary-electrophoresis analytical method by the indirect absorbance detecting method which measures the small ion of absorption without absorption on detection wavelength Two or more ultraviolet absorber ion is included as said background electrolyte. It is the capillary-electrophoresis analytical method which the effective mobility of those ultraviolet absorber ion differs usually, and is characterized by those effective mobility being close to the effective mobility of the ion kind for analysis of either of the samples respectively.

[translation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

DETAILED DESCRIPTION

Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to the analytical method by the indirect absorbance detecting method, especially about the analytical method by capillary electrophoresis. The indirect absorbance detecting method can be used for the separation analysis of low-molecular-weight ion.

[0002]

[Description of the Prior Art] The analysis using capillary-electrophoresis equipment, especially capillary zone-electrophoresis mode is briskly used as biopolymer analytical method. It was seldom used for analysis of low-molecular-weight ion like inorganic compound ion until now compared with it. It is because a problem is in separation since it is not used on pH of carrier electrolyte liquid also when it is because a problem is in detection absorption measurement and in respect of fluorescence since most low-molecular-weight ion does not have a chromophore in the 1st in the reason and is used on the indirect absorbance detecting method the 2nd, but mobility is approaching between sample ion kinds.

[0003] However, recent years come, a molar extinction coefficient is large as an anion of a background electrolyte, an anion, a cation, sugar, etc. are measured by the indirect UV detection which chose what has effective mobility close to the sample ion kind for analysis, and it is becoming clear that the capillary-electrophoresis analytical method by the indirect absorbance detecting method is useful as flexible analytical method (see the European Patent disclosure official report 442315A1, Journal of Chromatography, 470 (1989) 299-308, etc.).

[0004]

[Problem(s) to be Solved by the Invention] By the indirect absorbance detecting method, when the effective mobility of a background electrolyte is not in agreement with the effective mobility of sample ion, it happens that concentration distribution of a sample zone becomes unsymmetrical by migration distribution of a sample component, and the peak required has tailing and chlorofluorocarbon TINGU. The detection peak by the conventional indirect UV detecting method is roughly shown in drawing 2 (A). One kind of UV absorber ion is used as a background electrolyte, and the example when the effective mobility is close to the mobility of the sample component of a peak 3 is expressed. It becomes a peak with tailing in which the time amount which serves as a peak with big leading according to the diffusion to a travelling direction like a peak 1 although obtained as a configuration with a contrastive peak 3, so that both difference becomes large in the case of a sample [than the effective mobility of a background electrolyte] component with larger effective mobility, and moves to reverse in the case of a sample component with effective mobility smaller than the effective mobility of a background electrolyte was long, and the die length of a zone also spread like a peak 6.

[0005] Thus, with sample ion with the effective mobility which is separated from the effective mobility of a background electrolyte, therefore the problem to which height becomes low and sensibility becomes low when separation between the sample ion kinds which ionic equivalent conductivity approached worsens as a result of tailing and chlorofluorocarbon TINGU happening, or a peak spreads arose, the ultraviolet absorber ion of a background electrolyte needed to be changed according to the object for analysis. In the capillary-electrophoresis analytical method which used the indirect absorbance detecting method, this invention improves separation of the peak between the sample ion kinds which ionic equivalent conductivity approached, and aims at raising detection sensitivity.

[0006]

[Means for Solving the Problem] In this invention, the effective mobility of those ultraviolet absorber ion differs mutually including two or more ultraviolet absorber ion as a background electrolyte, and it is made respectively close [those

effective mobility] to the effective mobility of the ion kind for analysis of either of the samples.

[0007] It is desirable to use UV absorber ion as the main background electrolytes as carrier electrolyte liquid, and to choose what has a large molar extinction coefficient by low concentration as much as possible. It is important when that a sample component permutes a background electrolyte efficiently raises sensitivity. When the molar extinction coefficient of a background electrolyte is small, it is because the peak by UV absorber ion laps with an indirect absorption peak and may carry out a mask, when it is used by low concentration. Moreover, when the ionic strength of a background electrolyte is raised too much, in connection with the Joule's heat, the base line of the background may carry out a drift.

While the effective mobility of sample ion and UV absorber ion is approaching, distribution of a sample ion zone becomes small, the asymmetry of a peak also becomes small, and the high sensitivity measurement is possible.

[0008] As an example of a background electrolyte, if it arranges to descending of effective mobility, a group division can be carried out as follows. It is so small [effective mobility is so large that it is upwards, and] that it turns down.

* A nitric acid, a nitrous acid, a chromic acid, vanadium acid, and ** aromatic carboxylic acid (a phthalic acid, a benzoic acid, trimesic acid, para hydroxybenzoic acid, etc.)

* basic UV absorbers, such as an aromatic series sulfonic-acids, alkyl sulfonic-acids (benzenesulfonic acid, naphthalene sulfonic-acid, benzene disulfon acid, etc.), dodecylbenzenesulfonic acid, ** nicotinic-acid, and ** diethyl barbital acid ** hydrazole ** creatinine, -- use as a compound background electrolyte combining two or more sorts of these. A capillary may process making the direction of seepage flow into reverse etc., in order to carry out chemical modification, to make it the conditions which added additives, such as a triton X-100, and stopped seepage flow or to make migration time amount brief.

[0009]

Function] Drawing 1 (B) shows the example at the time of using UV absorber ion of two components as a background electrolyte, one of the effective mobility of UV absorber ion of the two components approaches the component ion of a peak 2, and another side is close to the component ion of a peak 5. Peaks 2 and 5 have a symmetry form, nothing and peaks 1 and 4 have chlorofluorocarbon TINGU slightly, and peaks 3 and 6 have tailing slightly.

[0010] Drawing 1 (C) has each effective mobility between the sample component of a peak 5, and the sample component of a peak 6 between the sample component of a peak 3, and the sample component of a peak 4 between the sample component of a peak 1, and the sample component of a peak 2 including UV absorber ion of three components as a background electrolyte. In this case, peaks 1-6 can acquire a sharp peak for a respectively almost symmetrical configuration over the range of nothing and large mobility.

[0011]

Example] Drawing 2 expresses roughly an example of the capillary zone-electrophoresis equipment with which this invention is applied. When a bore is 50-75 micrometers and a capillary 12 makes electrophoresis cause, the end is dipped in carrier electrolyte liquid 14, the other end is dipped in the carrier electrolyte liquid 16 of another side, and the migration electrical potential difference to about 30kV is impressed by the high voltage power supply 18 between both carrier electrolyte liquid. UV detector 22 is formed in the termination side of migration, and UV absorption of the ultraviolet absorber which migrates the inside of a capillary 12 is measured.

[0012] The example of separation of anion 9 component which used the barbital-HCl buffer and the sodium chromate for drawing 3 as a background electrolyte is shown. The analysis conditions are as follows.

background electrolyte: A 6mM barbital-HCl buffer and 2mM sodium-chromate additive: bromination -- tetradecyl trimethylammonium (pH8.4)

sample impregnation: It is application-of-pressure migration for 3 seconds at 30 gf/cm². : -24kV / 8microA capillary: the bore of 75 micrometers, the length of 40cm (overall length of 70cm) of the migration section

Detection wavelength: 240nm sample: Br- NO₂- NO₃- N₃- CO₃²⁻, C₃H₇COO- C₄H₉COO- C₅H₁₁COO-, (glutamic acid) - A barbital in this example CO₃²⁻, Br- with a sodium chromate perform indirect UV detection of carboxylic-acid ion and glutamic-acid ion, and large [mobility], NO₂- NO₃- Indirect UV detection of N₃- is governed.

[0013]

Effect of the Invention] Indirect detection of the ion kind of a field with the mobility of arbitration can be carried out at high sensitivity, without producing an unsymmetrical peak, since two or more sorts are used combining the thing respectively near the effective mobility of the ion kind for analysis of either of the samples with the ultraviolet absorber on which has various effective mobility as a background electrolyte in this invention.

ranslation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

TECHNICAL FIELD

[Industrial Application] This invention relates to the analytical method by the indirect absorbance detecting method specially about the analytical method by capillary electrophoresis. The indirect absorbance detecting method can be used for the separation analysis of low-molecular-weight ion.

[Translation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

PRIOR ART

Description of the Prior Art] The analysis using capillary-electrophoresis equipment, especially capillary zone-electrophoresis mode is briskly used as biopolymer analytical method. It was seldom used for analysis of low-molecular-weight ion like inorganic compound ion until now compared with it. It is because a problem is in separation since it is not used on pH of carrier electrolyte liquid also when it is because a problem is in detection absorption measurement and in spect of fluorescence since most low-molecular-weight ion does not have a chromophore in the 1st in the reason and is used on the indirect absorbance detecting method the 2nd, but mobility is approaching between sample ion kinds. [003] However, recent years come, a molar extinction coefficient is large as an anion of a background electrolyte, an ion, a cation, sugar, etc. are measured by the indirect UV detection which chose what has effective mobility close to the sample ion kind for analysis, and it is becoming clear that the capillary-electrophoresis analytical method by the indirect absorbance detecting method is useful as flexible analytical method (see the European Patent public presentation official report 0442315A1, Journal of Chromatography, 470 (1989) 299-308, etc.).

[translation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

EFFECT OF THE INVENTION

Effect of the Invention] Indirect detection of the ion kind of a field with the mobility of arbitration can be carried out at high sensitivity, without producing an unsymmetrical peak, since two or more sorts are used combining the thing respectively near the effective mobility of the ion kind for analysis of either of the samples with the ultraviolet absorber in which has various effective mobility as a background electrolyte in this invention.

[translation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

TECHNICAL PROBLEM

Problem(s) to be Solved by the Invention] By the indirect absorbance detecting method, when the effective mobility of a background electrolyte is not in agreement with the effective mobility of sample ion, it happens that concentration distribution of a sample zone becomes unsymmetrical by migration distribution of a sample component, and the peak required has tailing and chlorofluocarbon TINGU. The detection peak by the conventional indirect UV detecting method is roughly shown in drawing 2 (A). One kind of UV absorber ion is used as a background electrolyte, and the example when the effective mobility is close to the mobility of the sample component of a peak 3 is expressed. It becomes a peak with tailing in which the time amount which serves as a peak with big leading according to the diffusion to a travelling reaction like a peak 1 although obtained as a configuration with a contrastive peak 3, so that both difference becomes large in the case of a sample [than the effective mobility of a background electrolyte] component with larger effective mobility, and moves in the case of a sample component with effective mobility conversely smaller than the effective mobility of a background electrolyte was long, and the die length of a zone also spread like a peak 6.

[0005] Thus, with sample ion with the effective mobility which is separated from the effective mobility of a background electrolyte, therefore the problem to which height becomes low and sensibility becomes low when separation between the sample ion kinds which ionic equivalent conductivity approached worsens as a result of tailing and chlorofluocarbon TINGU happening, or a peak spreads arose, the ultraviolet absorber ion of a background electrolyte needed to be changed according to the candidate for analysis. In the capillary-electrophoresis analytical method which used the indirect absorbance detecting method, this invention improves separation of the peak between the sample ion kinds which ionic equivalent conductivity approached, and aims at raising detection sensitivity.

[translation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

MEANS

[Means for Solving the Problem] In this invention, the effective mobility of those ultraviolet absorber ion differs mutually including two or more ultraviolet absorber ion as a background electrolyte, and it is made respectively close [those effective mobility] to the effective mobility of the ion kind for analysis of either of the samples.

[0007] It is desirable to use UV absorber ion as the main background electrolytes as carrier electrolyte liquid, and to choose what has a large molar extinction coefficient by low concentration as much as possible. It is important when that a sample component permutes a background electrolyte efficiently raises sensibility. When the molar extinction coefficient of a background electrolyte is small, it is because the peak by UV absorber ion laps with an indirect absorption peak and may carry out a mask, when it is used by low concentration. Moreover, when the ionic strength of a background electrolyte is raised too much, in connection with the Joule's heat, the base line of the background may carry out a drift. While the effective mobility of sample ion and UV absorber ion is approaching, distribution of a sample ion zone becomes small, the asymmetry of a peak also becomes small, and the high sensitivity measurement is possible.

[0008] As an example of a background electrolyte, if it arranges to descending of effective mobility, a group division can be carried out as follows. It is so small [effective mobility is so large that it is upwards, and] that it turns down.

* A nitric acid, a nitrous acid, a chromic acid, vanadium acid, and ** aromatic carboxylic acid (a phthalic acid, a benzoic acid, trimesic acid, para hydroxybenzoic acid, etc.)

* basic UV absorbers, such as an aromatic series sulfonic-acids, alkyl sulfonic-acids (benzenesulfonic acid, naphthalene sulfonic-acid, benzene disulfon acid, etc.), dodecylbenzenesulfonic acid, ** nicotinic-acid, and ** diethyl barbital acid ** hydrazide ** creatinine, -- use as a compound background electrolyte combining two or more sorts of these. A capillary may process making the direction of seepage flow reverse etc., in order to carry out chemical modification, to make it the conditions which added additives, such as a triton X-100, and stopped seepage flow or to short-**** migration time amount.

[Translation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

PERATION

function] Drawing 1 (B) shows the example at the time of using UV absorber ion of two components as a background electrolyte, one of the effective mobility of UV absorber ion of the two components approaches the component ion of a peak 2, and another side is close to the component ion of a peak 5. Peaks 2 and 5 have a symmetry form, nothing and peaks 1 and 4 have chlorofluorocarbon TINGU slightly, and peaks 3 and 6 have tailing slightly.

010] Drawing 1 (C) has each effective mobility between the sample component of a peak 5, and the sample component of a peak 6 between the sample component of a peak 3, and the sample component of a peak 4 between the sample component of a peak 1, and the sample component of a peak 2 including UV absorber ion of three components as a background electrolyte. In this case, peaks 1-6 can acquire a sharp peak for a respectively almost symmetrical configuration over the range of nothing and large mobility.

Translation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

XAMPLE

Example] Drawing 2 expresses roughly an example of the capillary zone-electrophoresis equipment with which this invention is applied. When a bore is 50-75 micrometers and a capillary 12 makes electrophoresis cause, the end is dipped in carrier electrolyte liquid 14, the other end is dipped in the carrier electrolyte liquid 16 of another side, and the migration electrical potential difference to about 30kV is impressed by the high voltage power supply 18 between both carrier electrolyte liquid. UV detector 22 is formed in the termination side of migration, and UV absorption of the ultraviolet absorber which migrates the inside of a capillary 12 is measured.

[012] The example of separation of anion 9 component which used the barbital-HCl buffer and the sodium chromate for Figure 3 as a background electrolyte is shown. The analysis conditions are as follows.

Background electrolyte: A 6mM barbital-HCl buffer and 2mM sodium-chromate additive: bromination -- tetradecyl dimethylammonium (pH8.4)

Sample impregnation: It is pressurization migration for 3 seconds at 30 gf/cm². : -24kV / 8microA capillary: The bore of 5 micrometers, die length of 40cm (overall length of 70cm) of the migration section

Detection wavelength: 240nm sample: Br- NO₂- NO₃- N₃- CO₃²⁻, C₃H₇COO- C₄H₉COO- C₅H₁₁COO-, (glutamic acid) - A barbital in this example CO₃²⁻, Br- with a sodium chromate perform indirect UV detection of carboxylic-acid ion and glutamic-acid ion, and large [mobility], NO₂- NO₃- Indirect UV detection of N₃- is governed.

[translation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

Drawing 1] It is drawing showing the peak acquired, and what depends (A) on the conventional approach using the background electrolyte containing one kind of UV absorber ion, and (B) and (C) express the peak by this invention which contains two kinds and three kinds of UV detection object ion in a background electrolyte, respectively.

Drawing 2] It is the block diagram showing an example of the capillary zone-electrophoresis equipment with which this invention is applied.

Drawing 3] It is drawing showing the example of analysis of one example.

[translation done.]

NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

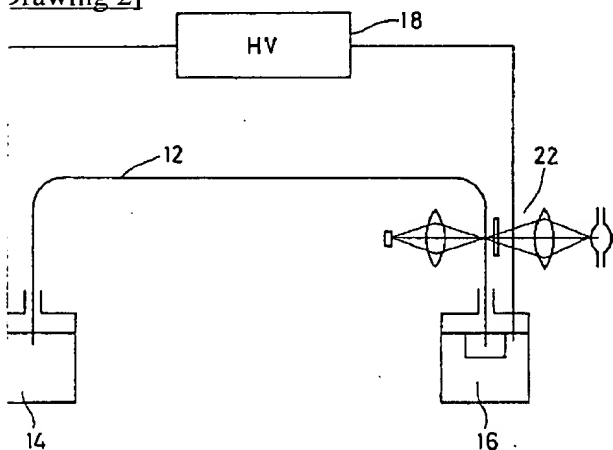
This document has been translated by computer. So the translation may not reflect the original precisely.

**** shows the word which can not be translated.

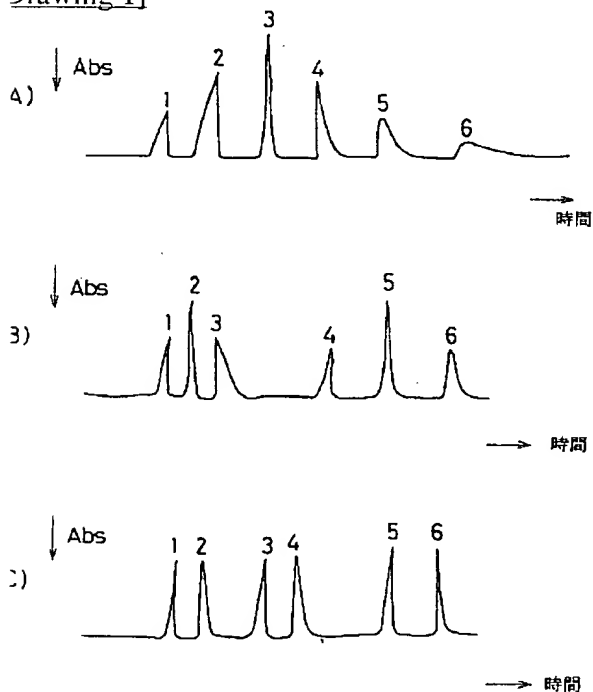
In the drawings, any words are not translated.

DRAWINGS

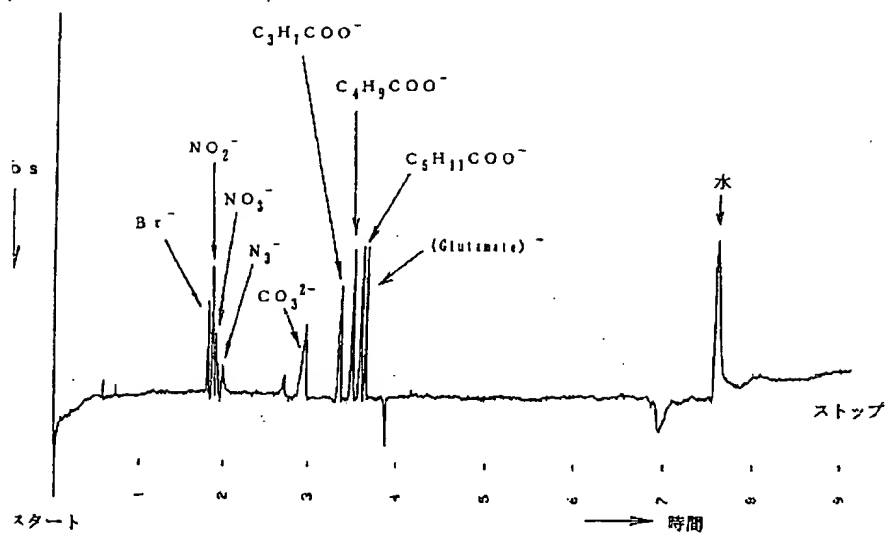
Drawing 2]



Drawing 1]



Drawing 3]



[translation done.]

(19)日本国特許庁(JP)

(12) 公開特許公報(A)

(11)特許出願公開番号

特開平6-82423

(43)公開日 平成6年(1994)3月22日

(51)Int.Cl.⁵

G 0 1 N 27/447

識別記号

片内整理番号

F I

技術表示箇所

7235-2J

G 0 1 N 27/ 26

3 3 1 Z

審査請求 未請求 請求項の数1(全 6 頁)

(21)出願番号

特願平4-257370

(22)出願日

平成4年(1992)8月31日

(71)出願人 000001993

株式会社島津製作所

京都府京都市中京区西ノ京桑原町1番地

(72)発明者 荒井 昭博

京都府京都市中京区西ノ京桑原町1番地

株式会社島津製作所三条工場内

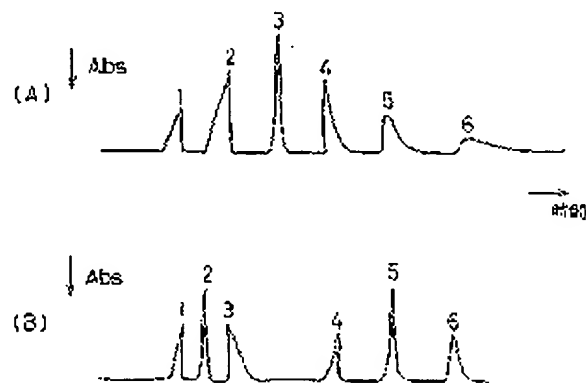
(74)代理人 弁理士 野口 繁雄

(54)【発明の名称】 間接吸光度検出法によるキャピラリ電気泳動分析方法

(57)【要約】

【目的】 試料イオン間のピークの分離をよくし、検出感度も高める。

【構成】 2成分のUV吸収体イオンをバックグラウンド電解質として用い、1つはピーク2の成分イオンに近接し、他方はピーク5の成分イオンに近接している場合(B)。ピーク2と5は対称形をなし、ピーク1と4はわずかにフロンティングをもち、ピーク3と6はわずかにテーリングをもち、バックグラウンド電解質として3成分のUV吸収体イオンを含み、それぞれの電荷移動度



【特許請求の範囲】

【請求項1】 紫外吸収検出器を備えたキャピラリー電気泳動装置を用い、キャリヤ電解質液に検出波長に吸収をもつバックグラウンド電解質を含み、検出波長に吸収をもたないか吸収の小さいイオンを測定する間接吸光度検出法によるキャピラリー電気泳動分析方法において、前記バックグラウンド電解質として複数の紫外線吸収体イオンを含み、それらの紫外線吸収体イオンの実効移動度は互いに異なり、かつそれらの実効移動度はそれぞれ試料中のいずれかの分析対象イオン種の実効移動度に近いものであることを特徴とするキャピラリー電気泳動分析方法。

【発明の詳細な説明】

【0001】

【産業上の利用分野】 本発明はキャピラリー電気泳動法による分析方法に関し、特に間接吸光度検出法による分析方法に関するものである。間接吸光度検出法は低分子量イオンの分離分析に利用することができる。

【0002】

【従来の技術】 キャピラリー電気泳動装置、特にキャピラリーゾーン電気泳動モードを用いた分析は、生体高分子分析方法として盛んに利用されている。それに比べて無機化合物イオンのような低分子量イオンの分析にはこれまであまり利用されていなかった。その理由は、第1に低分子量イオンの大部分は発色団をもたないので、吸収測定や発光性の点で検出に問題があるためであり、第2に間接吸光度検出法によるときもキャリヤ電解質液のpHによらず、試料イオン種間で移動度が接近しているため分離に問題があるためである。

【0003】 しかし、近年になってバックグラウンド電解質のアニオンとしてモル吸光係数が大きく、実効移動度が分析対象の試料イオン種に近いものを選んだ間接UV検出によりアニオン、カチオン、糖質などが測定され、間接吸光度検出法によるキャピラリー電気泳動分析方法は汎用性のある分析方法として有用であることが明らかになってきている（ヨーロッパ特許公開公報0442315A1やJournal of Chromatography, 470 (1989) 299-308などを参照）。

【0004】

【発明が解決しようとする課題】 間接吸光度検出法で

移動度より大きい試料成分の場合、両者の差が大きくなるほどピーク1のように進行方向への拡散による大きなリーディングをもつピークとなり、逆に実効移動度がバックグラウンド電解質の実効移動度より小さい試料成分の場合、移動する時間が長く、ピーク6のようにゾーンの長さも広がったテールリングをもつピークとなる。

【0005】 このように、バックグラウンド電解質の実効移動度から離れた実効移動度をもつ試料イオンではテールリングやフロンテイングが起こる結果、当量イオン伝導度の接近した試料イオン種間での分離が悪くなったり、ピークが広がることによって高さが低くなり感度が低くなる問題が生じる、そのため、分析対象に合わせてバックグラウンド電解質の紫外線吸収体イオンを変える必要があった。本発明は間接吸光度検出法を用いたキャピラリー電気泳動分析方法において、当量イオン伝導度の接近した試料イオン種間のピークの分離をよくし、検出感度も高めることを目的とするものである。

【0006】

【課題を解決するための手段】 本発明では、バックグラウンド電解質として複数の紫外線吸収体イオンを含み、それらの紫外線吸収体イオンの実効移動度は互いに異なり、かつそれらの実効移動度はそれぞれ試料中のいずれかの分析対象イオン種の実効移動度に近いものであるようにする。

【0007】 キャリヤ電解質液としてはUV吸収体イオンを主なバックグラウンド電解質とし、できるだけ低濃度でモル吸光係数の大きいものを選択することが望ましい。試料成分がバックグラウンド電解質を効率的に置換することが感度を上げるうえで重要である。バックグラウンド電解質のモル吸光係数が小さいときは、低濃度で使用した場合にUV吸収体イオンによるピークが間接吸収ピークと重なり、マスクすることがあるからである。また、バックグラウンド電解質のイオン強度を上げすぎると、ジュール熱にともないバックグラウンドのベースラインがドリフトすることがある。試料イオンとUV吸収体イオンの実効移動度が接近しているときに、試料イオンゾーンの分散が小さくなり、ピークの非対称性も小さくなって、最も高感度な測定が可能である。

【0008】 バックグラウンド電解質の具体例として

は、実効移動度の大きい順に並べると次のようにグルー

⑤イミダゾール

⑦クレアチニンなどの塩基性UV吸収体

これらの2種以上を組み合わせるバックグラウンド電解質として用いる。キャピラリは化学修飾したり、トリトンX-100などの添加剤を加えて視透流を抑えた条件にしたり、泳動時間を短くするために視透流の方向を逆にするなどの処理を施してもよい。

【0009】

【作用】図1(B)は2成分のUV吸収体イオンをバックグラウンド電解質として用いた場合の例を示したものであり、その2成分のUV吸収体イオンの実効移動度の1つはピーク2の成分イオンに近接し、他方はピーク5の成分イオンに近接している。ピーク2と5は対称形をなし、ピーク1と4はわずかにフロンティングをもち、ピーク3と6はわずかにテーリングをもっている。

【0010】図1(C)はバックグラウンド電解質として3成分のUV吸収体イオンを含み、それぞれの実効移動度がピーク1の試料成分とピーク2の試料成分の間、ピーク3の試料成分とピーク4の試料成分の間、ピーク5の試料成分とピーク6の試料成分の間にある。この場合、ピーク1～6はそれぞれほぼ対称的な形状をなし、広い移動度の範囲にわたってシャープなピークを得ることができる。

【0011】

【実施例】図2は本発明が適用されるキャピラリゾーン電気泳動装置の一例を概略的に表わしたものである。キャピラリ12は内径が50～75μmのもので、電気泳動を起こさせるときは、その一端がキャリヤ電解液14に浸され、他端が他方のキャリヤ電解液16に浸され、両キャリヤ電解液間に高圧電源18により約30KVまでの泳動電圧が印加される。泳動の終端側にはUV検出器22が設けられており、キャピラリ12中を泳動する紫外線吸収体のUV吸収が測定される。

【0012】図3にバルビタール-HClバッファとクロム酸ナトリウムとをバックグラウンド電解質として用*

*いたアニオン9成分の分離例を示す。分析条件は次の通りである。

バックグラウンド電解質： 6mMバルビタール-HClバッファ及び2mMクロム酸ナトリウム

添加剤： 臭化テトラデシルトリメチルアンモニウム(pH8.4)

試料注入： 30gf/cm²で3秒間加圧

泳動： -24KV/8μA

キャピラリ： 内径75μm、泳動部の長さ40cm(全長70cm)

検出波長： 240nm

サンプル： Br⁻、NO₂⁻、NO₃⁻、N₃⁻、CO₃²⁻、C₂H₃COO⁻、C₂H₅COO⁻、C₂H₁₁COO⁻、(グルタミン酸)⁻

この例ではバルビタールがCO₃²⁻、カルボン酸イオン及びグルタミン酸イオンの間接UV検出を行ない、クロム酸ナトリウムが移動度の大きいBr⁻、NO₂⁻、NO₃⁻、N₃⁻の間接UV検出を支配している。

【0013】

【発明の効果】本発明ではバックグラウンド電解質として様々な実効移動度をもつ紫外線吸収体イオンでそれぞれ試料中のいずれかの分析対象イオン種の実効移動度に近いものを複数種組み合わせることで、非対称ピークを生じることなく、任意の移動度をもつ領域のイオン種を高感度に間接検出することができるようになる。

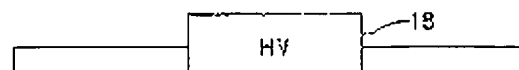
【図面の簡単な説明】

【図1】得られるピークを示す図であり、(A)は一種類のUV吸収体イオンを含むバックグラウンド電解質を用いた従来の方法によるもの、(B)と(C)はそれぞれ2種類と3種類のUV検出体イオンをバックグラウンド電解質に含む本発明によるピークを表わしたものである。

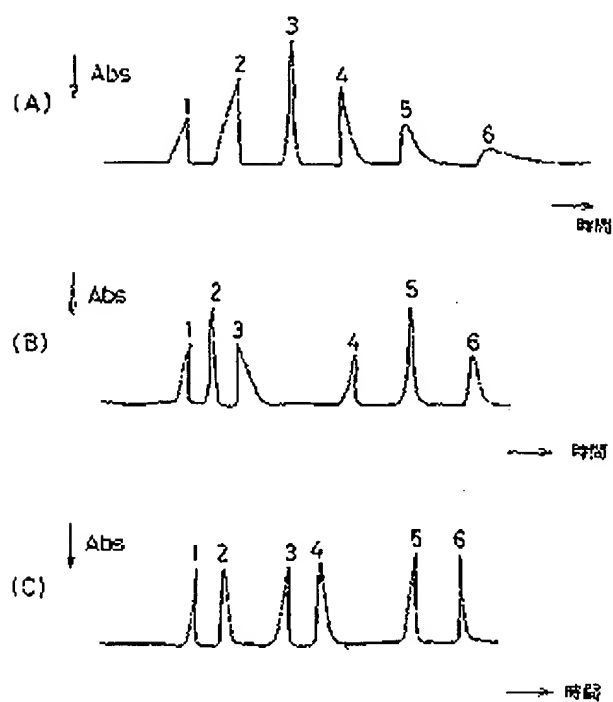
【図2】本発明が適用されるキャピラリゾーン電気泳動装置の一例を示す構成図である。

【図3】一実施例の分析例を示す図である。

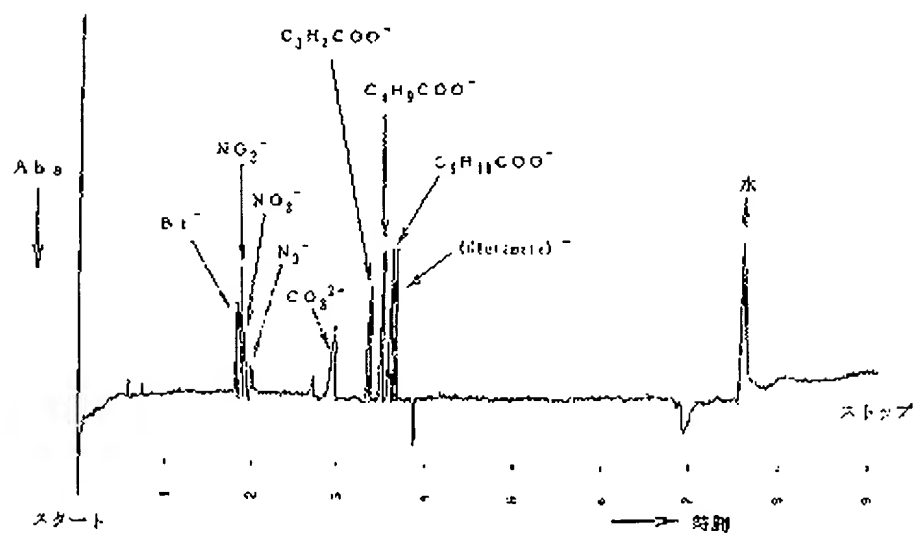
【図2】



【図1】



【図3】



【手続補正2】

【補正対象書類名】明細書

【補正対象項目名】0003

【補正方法】変更

【補正内容】

【0003】しかし、近年になってバックグラウンド電解質イオンとしてモル吸光係数が大きく、実効移動度が分析対象の試料イオン程に近いものを選んだ間接UV検出によりアニオン、カチオン、糖質などが測定され、間接吸光度検出法によるキャピラリー電気泳動分析方法は汎用性のある分析方法として有用であることが明らかになってきている（ヨーロッパ特許公開公報0442315A1やJournal of Chromatography, 470 (1989) 299-308などを参照）。

【手続補正3】

【補正対象書類名】明細書

【補正対象項目名】0005

【補正方法】変更

【補正内容】

【0005】このように、バックグラウンド電解質の実効移動度から離れた実効移動度をもつ試料イオンではテーリングやフロンテイングが起こる結果、当量イオン伝導度の接近した試料イオン種間での分離が悪くなったり、ピークが広がることによって高さが低くなり感度が低くなる問題が生じる、そのため、分析対象に合わせてバックグラウンド電解質の紫外線吸収イオンを変える必要があった。本発明は間接吸光度検出法を用いたキャピラリー電気泳動分析方法において、移動度の離れているイオン種間のピーク形状を改善するとともに当量イオン伝導度の接近した試料イオン種間のピークの分離をよくし、検出感度も高めることを目的とするものである。

【手続補正4】

【補正対象書類名】明細書

【補正対象項目名】0006

【補正方法】変更

【補正内容】

【0006】

【課題を解決するための手段】本発明では、バックグラウンド電解質として複数の紫外線吸収イオンを含み、それらの紫外線吸収イオンの実効移動度は互いに異なり、かつ

のを選択することが望ましい。試料成分がバックグラウンド電解質と効率的に置換することが感度を上げるうえで重要であり、バックグラウンド電解質のモル吸光係数が小さいときは、低濃度で使用的場合にUV吸収イオンによるピークが間接吸収ピークと重なり、マスクすることがあるからである。また、バックグラウンド電解質のイオン強度を上げすぎると、ジュール熱にともない試料ゾーンの広がりが起こったりバックグラウンドのベースラインがドリフトすることがある。試料イオンとUV吸収イオンの実効移動度が接近しているときに、試料イオンゾーンの分散が小さくなり、ピークの非対称性も小さくなって、最も高感度な測定が可能である。

【手続補正6】

【補正対象書類名】明細書

【補正対象項目名】0009

【補正方法】変更

【補正内容】

【0009】

【作用】図1(B)は2成分のUV吸収イオンをバックグラウンド電解質として用いた場合の例を示したものであり、その2成分のUV吸収イオンの実効移動度の1つはピーク2の成分イオンに近接し、他方はピーク5の成分イオンに近接している。ピーク2と5は対称形をなし、ピーク1と4はわずかにフロンテイングをもち、ピーク3と6はわずかにテーリングをもっている。

【手続補正7】

【補正対象書類名】明細書

【補正対象項目名】0010

【補正方法】変更

【補正内容】

【0010】図1(C)はバックグラウンド電解質として3成分のUV吸収イオンを含み、それぞれの実効移動度がピーク1の試料成分とピーク2の試料成分の間、ピーク3の試料成分とピーク4の試料成分の間、ピーク5の試料成分とピーク6の試料成分の間にある。この場合、ピーク1～6はそれぞれほぼ対称的な形状をなし、広い移動度の範囲にわたってシャープなピークを得ることができる。

【手続補正8】

【補正対象書類名】明細書

(6)

特開平6-82423

試料注入: 30 gf/cm^2 で3秒間加圧
泳動: $-24\text{ KV}/8\text{ }\mu\text{A}$
キャピラリ: 内径 $75\text{ }\mu\text{m}$ 、泳動部の長さ 40 cm
(全長 70 cm)
検出波長: 240 nm
サンプル: Br^- , NO_2^- , NO_3^- , N_3^- ,

CO_3^{2-} , $\text{C}_2\text{H}_3\text{COO}^-$, $\text{C}_4\text{H}_9\text{COO}^-$, $\text{C}_6\text{H}_{11}\text{COO}^-$, (グルタミン酸) $^-$
この例ではバルビタールが CO_3^{2-} 、カルボン酸イオン及びグルタミン酸イオンの間接UV検出を行ない、クロム酸ナトリウムが移動度の大きい Br^- , NO_2^- , NO_3^- , N_3^- の間接UV検出を支配している。